Abstract

Antiplatelet agents are mainly used in the prevention and management of atherothrombotic complications. Dual antiplatelet therapy, combining aspirin and clopidogrel, is the standard care for patients having acute coronary syndromes or undergoing percutaneous coronary intervention according to the current ACC/AHA and ESC guidelines. But in spite of administration of dual antiplatelet therapy, some patients develop recurrent cardiovascular ischemic events especially stent thrombosis which is a serious clinical problem. Antiplatelet response to clopidogrel varies widely among patients based on ex vivo platelet function measurements. Clopidogrel is an effective inhibitor of platelet activation and aggregation due to its selective and irreversible blockade of the P2Y12 receptor. Patients who display little attenuation of platelet reactivity with clopidogrel therapy are labeled as low or nonresponders or clopidogrel resistant. The mechanism of clopidogrel resistance remains incompletely defined but there are certain clinical, cellular and genetic factors including polymorphisms responsible for therapeutic failure. Currently there is no standardized or widely accepted definition of clopidogrel resistance. The future may soon be realised in the routine measurement of platelet activity in the same way that blood pressure, cholesterol and blood sugar are followed to help guide the therapy, thus improving the care for millions of people. This review focuses on the methods used to identify patients with clopidogrel resistance, the underlying mechanisms, metabolism, clinical significance and current therapeutic strategies to overcome clopidogrel resistance.

Key words: Acute coronary syndrome; Percutaneous coronary intervention; Clopidogrel; Platelet aggregation

Introduction

Oral antiplatelet agents are the cornerstone of modern pharmacotherapy in the prevention and management of cardiovascular atherothrombotic diseases according to the current ACC/AHA and ESC guidelines.1,2 It is well-established that the antiplatelet response to clopidogrel is not uniform and it varies widely among patients3,4 and it reflects failure of clopidogrel to achieve its anti-aggregatory effect. When combined with aspirin, clopidogrel is the gold standard for the prevention of subacute stent thrombosis (SAT) in subjects undergoing PCI and thus reducing major adverse cardiovascular events in patients with non-ST segment elevation acute coronary syndromes.5 This article discusses the definition, detection, risk factors and clinical consequences of clopidogrel resistance and role of current therapeutic strategies to overcome clopidogrel resistance.

Platelet aggregation

Platelets adhere to the sites of vascular injury. Adenosine diphosphate (ADP) plays an important role in the platelet activation and aggregation.6 As a result of damaged or disrupted endothelium, circulating platelets adhere to the vessel wall through interactions with von Willebrand factor, collagen, fibronectin, vitronectin, laminin etc.7 After activation, platelets

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release secondary agonists like thromboxane A2 and ADP or lead to the thrombin formation locally as a result of coagulation cascade. Additionally platelets aggregate to the adherent platelet layer through cross linking of fibrinogen to adjacent platelets creating a platelet mass which acts as a scaffold for coagulation. ADP binds to neighboring platelets through two G-protein-coupled receptors (P2Y1 and P2Y2) and the cation channel coupled P2X1 receptor. ADP-induced platelet aggregation occurs as a result of stimulation of both P2Y1 and P2Y2 receptors. Activated and degranulated platelets expose glycoprotein (GP) IIb/IIIa receptors at their surface thereby allowing fibrinogen binding. Thus platelets activation occurs as a result of formation of bridges between adjacent activated platelets.

**Pharmacokinetics of clopidogrel**

Clopidogrel is an ADP receptor antagonist like all thienopyridines and is an inactive prodrug. It requires oxidation by the hepatic cytochrome P450 (CYP 450) to generate an active thiol metabolite. Esterases hydrolyze clopidogrel into an inactive carboxylic acid derivative which constitutes 85% of clopidogrel-related circulating compounds. CYP 3A4 and CYP3A5 are the enzymes responsible for the oxidation of the thiophene ring of clopidogrel to 2-oxoclopidogrel which is further oxidized and ultimately leads to formation of carboxyl and thiol group. The thiol group forms a disulfide bridge with two extracellular cysteine residues located on the platelet surfaces and this causes irreversible ADP blockade. Many studies have documented that CYP2C19 genotyping detects more than 90% of poor clopidogrel metabolizers. The carriers of CYP2C19 3 and 4 alleles may also result in no enzymatic activity in the same way as CYP2C19 2. Polymorphisms in carriers of CYP2C9 gene may be responsible for reduced clopidogrel metabolism. The existence of CYP2C19 polymorphisms may be responsible for increased cardiovascular events and all cause mortality as shown in various studies. A standard dose of clopidogrel will achieve P2Y12 antagonism, which translates into about 50% of the inhibitors of ADP-induced platelet aggregation. In healthy subjects platelet inhibition is dose-related up to a single dose of 400 mg with no further increase with 600 mg. The maximum inhibition achieved with a single 400 mg dose takes 2 to 5 hours while a daily dose of 75 mg takes 3–7 days to reach the same level of inhibition. Clopidogrel also attenuates platelet-leukocyte aggregate formation, levels of CRP, P selectin, CD40L and the rate of thrombin formation.

**Mechanisms of clopidogrel resistance**

**Drug-drug interactions**

In a study by Gilard et al it has been shown that omeprazole as proton pump inhibitor (PPI) decreased the clinical efficacy of clopidogrel using the vasodilator-stimulated phosphoprotein (VASP) method. Cuisset et al in their study involving subjects who underwent coronary stenting for non-ST elevation ACS have shown that the number of clopidogrel nonresponders in the omeprazole group was more as compared to pantoprazole group. Both clopidogrel and PPIs are metabolized by the same CYP 450 pathway but the diminished biological effect of clopidogrel may be due to competitive effect of PPI on the CYP2C19 enzyme. COGENT trial involving randomized 3627 patients with ACS and/or stent placement on clopidogrel found that omeprazole group had few gastrointestinal events unlike the placebo group. Previously statins have been shown to reduce clopidogrel’s efficacy possibly due to the common sharing CYP3A4 enzymatic pathway between clopidogrel and statins. But recent studies have shown that use of statins with high dose clopidogrel (600 mg) is safe. Gurbel et al have demonstrated that decreased response to clopidogrel may occur with high dose of calcium channel blockers and angiotensin converting enzyme inhibitors.

**Definition of clopidogrel resistance**

Clopidogrel resistance is a phenomenon encountered in daily medical practice. In its broadest sense, resistance refers to the continued chance of ischemic events despite adequate clopidogrel therapy and compliance (ie, P2Y2 receptors of the platelet). However, the term nonresponders seems more appropriate given the fact that patients retain a degree of response to the medical treatment. Other terms have been used to describe patients with ineffective platelet inhibition by clopidogrel as hyporesponsive, non-responsive and semi-responsive.
The prevalence of clopidogrel nonresponse in patients varies between 4–30% among different populations 24 hours after administration. The reported rates vary depending upon the different tests used to measure the extent of ADP-induced platelet aggregation and the presence of factors contributing to greater baseline platelet reactivity.

Potential mechanisms of clopidogrel resistance are as follows:

I. Extrinsic mechanisms
   1. Patient non-compliance
   2. Underdosing or inappropriate dosing of clopidogrel
   3. Drug-drug interaction involving CYP3A4

II. Intrinsic mechanisms
   1. Genetic variables
      (a) Polymorphisms of P2Y12 receptors
      (b) Polymorphisms of CYP3A5
   2. Increased release of ADP
   3. Alternative pathways of platelet activation
      (a) Failure to inhibit catecholamine-mediated platelet activation (epinephrine)
      (b) Up-regulation of P2Y12 – independent pathways (thrombin, thromboxin A2, collagen).

High pretreatment platelet reactivity and thrombotic burden may cause diminished response to clopidogrel therapy because these patients remain the most reactive during the first five days of clopidogrel treatment. Overweight and obesity are also associated with lower clopidogrel platelet effect. But the limitation of this study was small size of sample and nonrandomized study design. So, these data need to be confirmed further with large scale studies.

**Laboratory methods to measure clopidogrel resistance**

Various laboratory methods to measure clopidogrel resistance have been demonstrated to assess ADP-induced platelet function such as turbidometric aggregation, flow cytometry to measure p-selectin and activated GP IIb/IIIa expression and vasodilator-stimulated phosphoprotein phosphorylation levels and point of care methods. Now R P2Y12 assay, platelet mapping with thromboelastography and multiplate analyzer are used. As clopidogrel induces platelet disaggregation, the response to clopidogrel would be better shown by measuring late platelet aggregation at six minutes after stimulation with ADP rather than maximum aggregation. The phosphorylation state of vasodilator-stimulated phosphoprotein is a specific intracellular marker of residual P2Y12 receptor reactivity in patients treated with clopidogrel and this technique is the most specific indicator of residual P2Y12 activity in patients treated with a P2Y12 inhibitor.

The phosphorylation of VASP is stimulated by the prostaglandin (PGE1) via the increase in platelet cAMP level leading to increase in platelet cAMP level. However, ADP inhibits PGE1-stimulated VASP phosphorylation by lowering the cAMP level through its effects on the P2Y12 receptors. Clopidogrel does not alter the basal and PGE1-stimulated VASP phosphorylation but strongly attenuates the inhibitory effect of ADP on PGE1-stimulated VASP phosphorylation.

**Clinical relevance of clopidogrel responsiveness**

Many studies have demonstrated that inadequate platelet inhibition leads to adverse clinical outcomes including recurrent ischemic cardiovascular events, stent thrombosis and periprocedural myocardial infarction. These studies have been done in different subgroups of patients undergoing PCI for ST elevation myocardial infarction (STEMI) or non-STEMI as well as elective PCI procedures, as documented in Table I.

**Management of clopidogrel resistance**

There is a clearcut relationship between failed clopidogrel therapy and cardiovascular events. Clinical approaches to overcome clopidogrel resistance have not yet been defined. However, different methods have been employed to overcome low response to clopidogrel. These include higher loading and/or maintenance dose or switching over to other antiplatelet drugs. An initial approach should be to correct the clinical factors responsible for resistance, ensuring proper patient compliance, to avoid or minimize drug-drug interactions and to achieve optimal control of glucose and cholesterol level.
Higher doses

In recent clinical studies of patients undergoing PCI, a loading dose of 600 mg clopidogrel was associated with a higher level of platelet inhibition, which means lower post-treatment reactivity to ADP and a lower incidence of nonresponsiveness when compared to 300 mg dose. Kastrati et al. in their study found that patient achieved additional platelet inhibition when a 75 mg/day clopidogrel maintainence dose was followed by an additional 600 mg loading dose. In the CLEAR PLATELET study, 600 mg loading dose showed a superior pharmacodynamic antiplatelet profile as compared to 300 mg clopidogrel loading dose. In the ISAR–CHOICE study, there was no additional effect regarding clopidogrel metabolite levels and platelet inhibition between the 600–900 mg loading dose possibly because of intestinal absorption concerned with response variability. So the 600 mg doses appear to achieve maximum inhibition more rapidly than 300 mg dose. Thus higher loading dose may be considered for selected patient showing high platelet reactivity to ADP. However the current ACC/AHA guidelines for PCI provide a class IIa recommendation that ‘a regimen of greater than 300 mg is reasonable to achieve higher levels of antiplatelet activity more rapidly’. Finally the ACC/AHA guidelines for PCI provide a class IIb

<table>
<thead>
<tr>
<th>Study</th>
<th>Number</th>
<th>Population</th>
<th>Results</th>
<th>Clinical relevance</th>
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<tr>
<td>45</td>
<td>60</td>
<td>Primary PCI for STEMI</td>
<td>↑ ADP-induced platelet aggregation (4th quartile)</td>
<td>Recurrent cardiovascular events</td>
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<tr>
<td>46</td>
<td>106</td>
<td>PCI for NSTEMI</td>
<td>↑ platelet aggregation</td>
<td>Recurrent cardiac events</td>
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<td>47</td>
<td>192</td>
<td>Non-emergent PCI</td>
<td>↑ periprocedural platelet aggregation</td>
<td>Post-PCI ischemic events (6 months)</td>
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<td>48</td>
<td>100</td>
<td>Non-emergent PCI</td>
<td>↑ Periprocedural platelet aggregation in patients on chronic clopidogrel</td>
<td>Post-PCI ischemic events (6 months)</td>
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<td>49</td>
<td>802</td>
<td>Elective PCI</td>
<td>↑ Periprocedural platelet aggregation</td>
<td>MACE distribution in the quartiles</td>
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<tr>
<td>50</td>
<td>36</td>
<td>Monitoring of VASP</td>
<td>↑ P2Y12 reactivity ratio (VASP levels)</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>51</td>
<td>120</td>
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<td>↑ P2Y12 reactivity ratio, platelet-stimulated GP2b/3a clopidogrel/ aspirin resistant patients</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>52</td>
<td>120</td>
<td>Elective PCI</td>
<td>↑ clopidogrel/aspirin resistant patients</td>
<td>Post PCI myocardial necrosis</td>
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<td>53</td>
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<td>↑ shear-induced platelet aggregation</td>
<td>Stent thrombosis</td>
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<tr>
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<td>↑ ADP-induced platelet reactivity</td>
<td>Stent thrombosis</td>
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<tr>
<td>56</td>
<td>160</td>
<td>PCI (STEMI excluded)</td>
<td>↑</td>
<td>Cardiac events</td>
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recommendation that in patient whom subacute thrombosis may be lethal, platelet aggregation studies may be considered and dose of clopidogrel increased to 150 mg/day if less than 50% inhibition of platelet aggregation is exhibited. However, the later guidelines do not support the cutpoint of 50% inhibition. But Bonello et al in their study involving 600 mg loading dose of clopidogrel found better clinical outcome in post-PCI patients where clopidogrel resistance and the platelet monitoring were done by vasodilator-stimulated phosphoprotein (VASP). But in the GRAVITUS trial done in PCI with drug eluting stents, clopidogrel resistant patients did not show any superiority of 150 mg over 75 mg of clopidogrel. But the ALBION study has also shown the superiority of loading dose of clopidogrel in the clopidogrel resistant patients. In the ARMYDA-4 study, reloading with 600 mg clopidogrel pre-PCI did not offer any additional benefit in patients on chronic clopidogrel therapy. In the ARMYDA-5 study, which compared clopidogrel loading with 600 mg ‘in lab’ vs 4–8 hours pre-PCI, did not show any significant difference between the two groups.

**Thienopyridine agents**

Other medications such as prasugrel, cangrelor, ticagrelor, cilostazole, AZ D6140 are also available for the treatment in clopidogrel resistance for ACS.

a) Prasugrel: It is a prodrug and irreversible inhibitor of P2Y12 with a rapid onset of action and produces more potent receptor blockade. It is mainly metabolized by cytochrome enzyme CYP3A and CYP2B6, lesser metabolized by CYP2C9 and CYP2C19. Inhibitor of platelet aggregation has been shown to be more rapid with prasugrel with the lesser incidence of nonresponders, compared with the standard clopidogrel dose of 75 mg. Vivott et al in their study involving 201 post-PCI patients has shown that prasugrel in loading dose (LD) of 60 mg and 10 mg maintenance dose (MD) achieved higher and more consistent level of platelet inhibition than clopidogrel at 600 mg LD and 150 mg MD. The ACAPULCO study has shown the superiority of prasugrel in platelet inhibition compared to high dose clopidogrel 900 mg LD or 150 mg MD. In the JUMBO-TIMI-26 trial, prasugrel treatment was associated with primary endpoint of regimen and bleeding events were the same for all doses of prasugrel. In the TRITON-TIMI-38 study, clinical outcome in terms of cardiovascular death, myocardial infarction and stroke were better with prasugrel compared with clopidogrel.

b) Cangrelor: It is a nonthienopyridine P2Y12 inhibitor given intravenously to overcome clopidogrel resistance. It has greater platelet inhibition than clopidogrel, rapid onset of action, short plasma half-life and is not activated by hepatic p450 cytochrome system and is a direct antagonist of P2Y12 receptor.

c) Cilostazole: It is a potent inhibitor of phosphodiesterase (PDE), targeting both platelets and vascular smooth muscle cells. It is found more potent than standard dose clopidogrel in studies using the Verify Now Assay. ACCEL-RESISTABCE study has shown that addition of cilostazole to standard clopidogrel proves more effective for platelet inhibition as compared to high maintenance dose of clopidogrel (150 mg/day). So cilostazole can be used in clopidogrel resistance.

d) Ticagrelor: It targets ADP receptors P2Y12. But unlike clopidogrel and prasugrel, the receptor inhibition is reversible. The PLATO trial involving 18624 patients with acute coronary syndrome taking ticagrelor (180 mg loading dose, 90 mg twice daily thereafter orally) and clopidogrel (300–600 mg loading dose, 75 mg daily thereafter) has shown that cardiovascular events were significantly low in the ticagrelor group but there was higher rate of major bleeding which was not related to coronary artery bypass grafting.

Various studies have failed to show the superiority of cangrelor over clopidogrel. There is need for further studies to prove the superiority of cangrelor in order to overcome clopidogrel resistance.

**Conclusion**

The use of clopidogrel has tremendously increased over the last few years, following its effectiveness together with aspirin in greatly reducing clinical adverse events in patients having acute coronary syndrome or undergoing PCI. There is no doubt that the laboratory phenomenon of “resistance” to clopidogrel exists.
There are many tests to assess platelet reactivity and these have shown a large variability in the response to clopidogrel therapy. Different methods report different prevalences depending upon the tests used, the cut-off value used to define resistance, the timing with respect to medication and population group studied. None of the methods used to assess platelet function fulfils the ideal criteria. There is a need to have a simple affordable, near-patient test useful in the clinical (not just laboratory) setting which should be validated in a large clinical trial in order to identify patients having clopidogrel resistance. However, use of higher loading or maintenance doses of clopidogrel or new and more potent P2Y12 receptor blockers is a potential alternative strategy although potential beneficial effects need to be balanced with an increased risk of bleeding. In addition to other factors, genetic polymorphisms and the patient risk profile should also be taken into account to detect clopidogrel resistance. Combined appropriate antiplatelet therapies may be required for the pharmacologic management of patients of high risk for arterial thrombotic events but not as a primary prevention modality or as an alternative to anticoagulants.

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